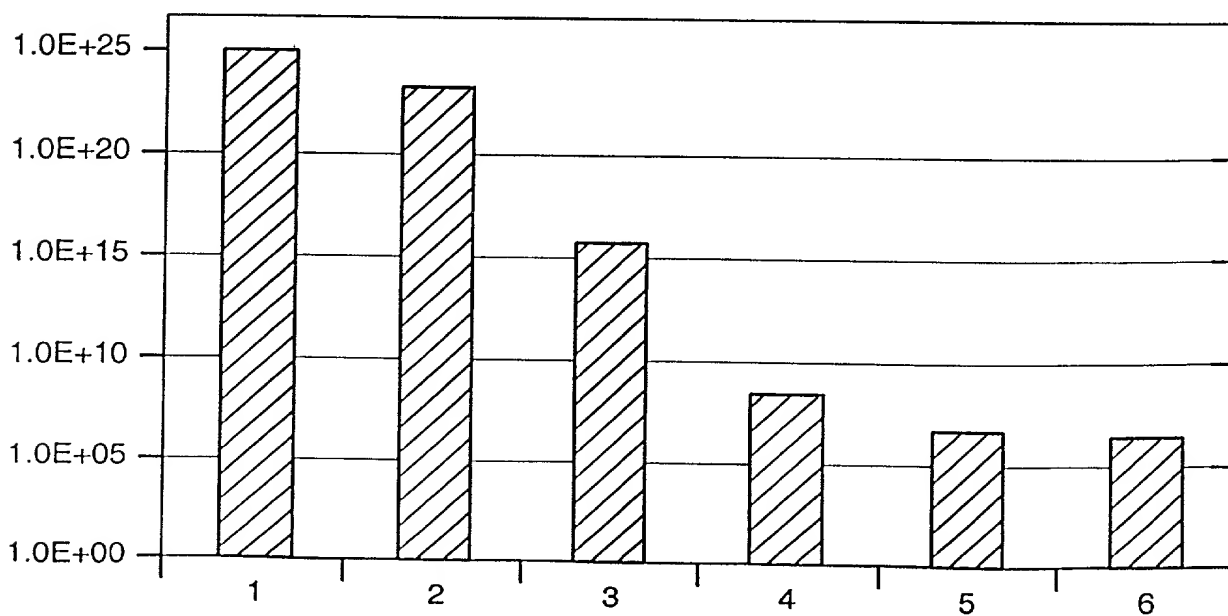


**FIG. 1**



**FIG. 2**

09782004 034201  
T02T20 40022/60



**FIG. 3**

FOOTED 40028/60

+

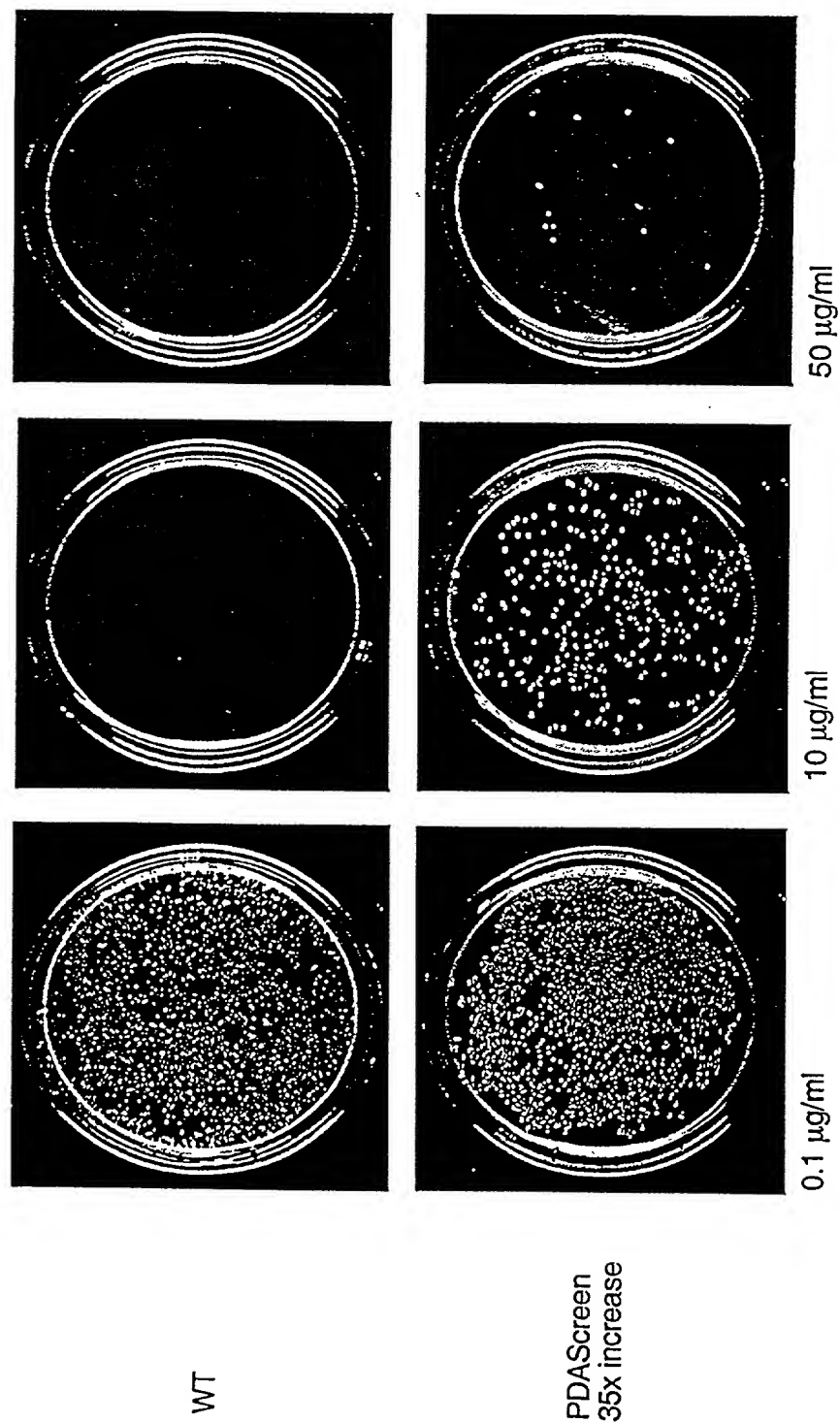
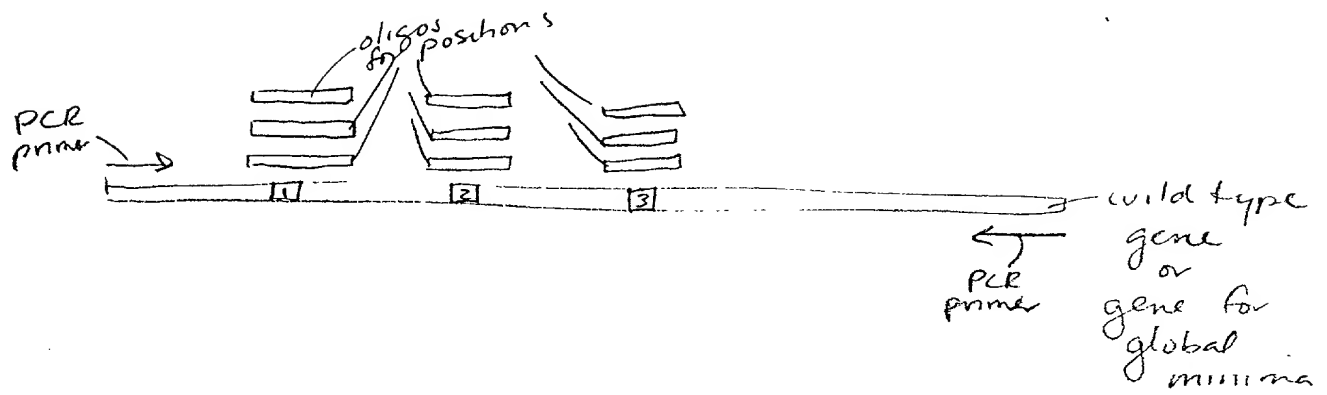


FIG. 4

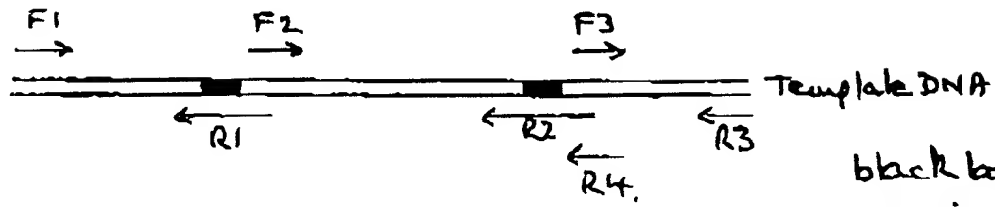
+

Figure 5



09/2004 00:00

# DIAGRAM

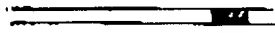


black box  
= region to  
be mutated.

Step 1: Set up 3 PCR reactions.

Products:

Tube 1:



Tube 2:



Tube 3:

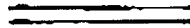
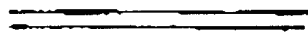
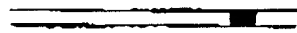


Fig 6A

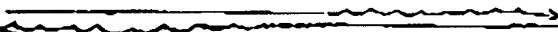
Step 2: Set up PCR reaction with products of tube 1 + products tube 2 + F1 + R4



Heat + anneal phase of PCR,



synthesis phase of PCR.



amplification phase, using F1 + R4.



during subsequent cycles.

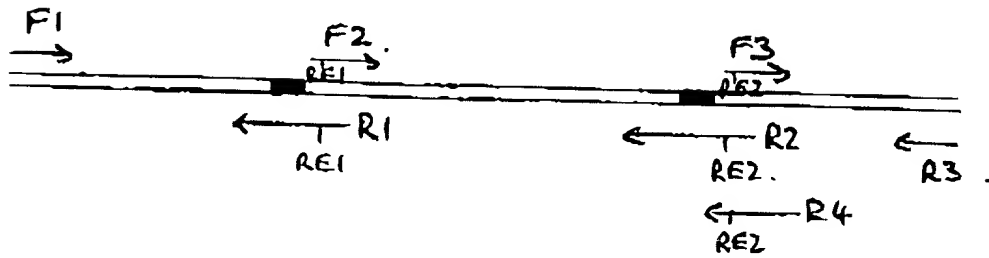
Step 3

Repeat step 2 using product from step 2 + product from Step 1, tube 3 + primers F1 + R3.

Fig 6B

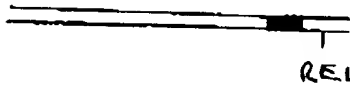
## DIAGRAM 2

Fig 7A

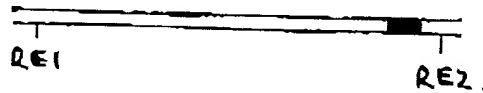


Step 1 Set up 3 PCR reactions:

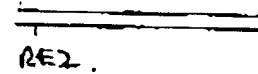
Tube 1:



Tube 2:



Tube 3:



Step 2: digest products from Step 1 with suitable restriction endonucleases

Step 3: ligate digested product from Step 2, Tube 2 with digested product from Step 2, Tube 1.

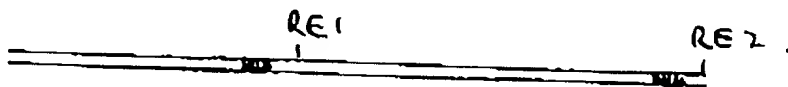


Fig 7B

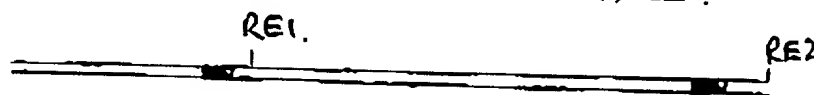
Step 4

Amplify <sup>via PCR</sup> ligated products of Step 3 with F1 + R4.



Step 5

Digest amplified product of step 4 with restriction endonuclease #2.



Step 6

Ligate product from Step 5 with product from Step 2, tube 1.



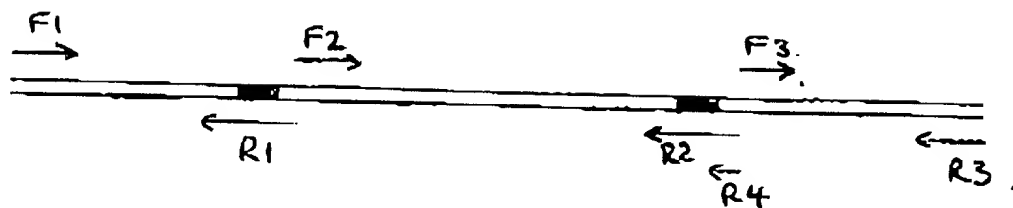
Step 7

Amplify product from step 6 with F1 + R3



Diagram 3

Fig 8



# Amplification Scheme Based on M13 Single Stranded Template

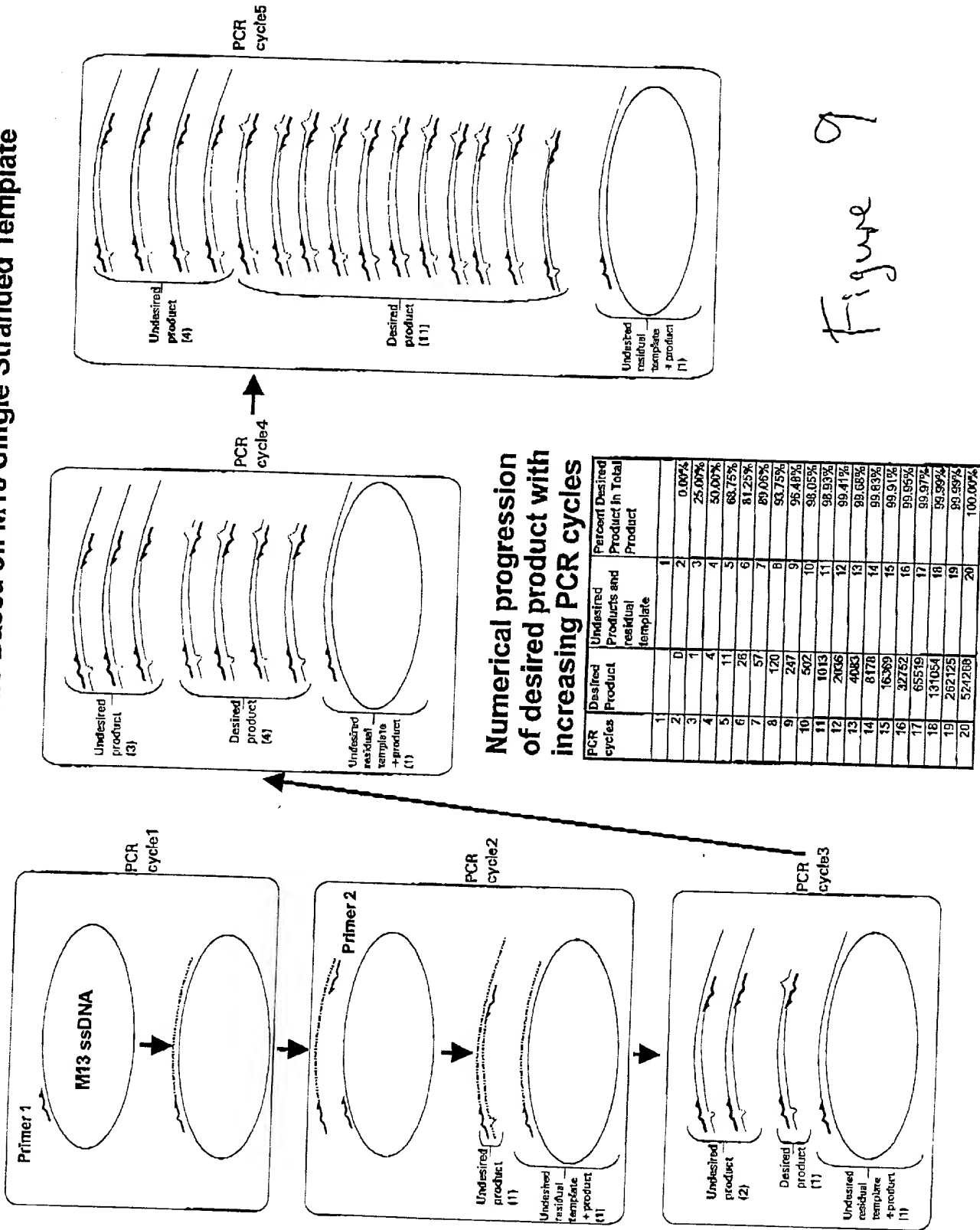


Figure 9